

### REMARKS

The Office Action mailed December 7, 2004 has been carefully reviewed and the foregoing amendments are made in response thereto. Claims 20, 22, 24-33, 35-40 and 42-47 are pending in the application. In view of the amendments and the following remarks, Applicants respectfully request reconsideration and reexamination of this application.

#### *Rejection under 35 USC § 112, second paragraph*

In paragraphs 4, claims 25, 29 and 33 have been rejected because the term "the adaptor-ligated fragments" lacks proper antecedent basis. Claims 25, 29 and 33 have been amended to provide antecedent basis for the phrase "adaptor-ligated fragments".

#### *Rejections under 35 USC § 103(a)*

In paragraph 6, the Examiner has rejected claims 20, 22, 24-27, 33, 40, 43 and 46 over McCasky Feazel *et al.* (U.S. Patent No. 6,100,030) in view of Pedersen (US 2003/0113737). Applicants traverse this rejection because the references fail to teach all of the claim limitations as required and even if the references did teach all the limitations there would be no motivation to combine the method taught by McCasky Feazel *et al.* with ligation of a 5' blocked adaptor and a 3' blocked adaptor.

Neither McCasky Feazel *et al.* nor Pedersen teaches ligation of a first and second adaptor to a restriction fragment where one adaptor is blocked from ligation to the fragment at the 3' end while the second adaptor is blocked from ligation at the 5' end. Pederson teaches only that adaptors can be blocked from ligation at either the 5' or 3' end or both, but does not teach or suggest the combination of a pair of adaptors where one is blocked at the 5' end and the other at the 3' end. The combination of blocking ligation to the fragments at the 5' end of one adaptor and the 3' end of the adaptor results in only one adaptor-ligated product that can be exponentially amplified (as shown in Fig. 1 of the instant application) and there is no teaching or suggestion of this in either McCasky Feazel *et al.* or Pedersen. Pedersen teaches only that you can block linkers from ligation on the 5' or 3' ends or both, but offers no teaching or suggestion of the specific combination of a first adaptor blocked from ligation at the 5' end and a second adaptor blocked from ligation at the 3' end.

The Examiner's assertion appears to be that because adaptors can be blocked from ligation on either the 5' or 3' ends that it would be prima facie obvious to modify the method of McCasky Feazel *et al.* by blocking ligation of one adaptor at the 5' end and one adaptor at the 3' end and that one of skill in the art would have been motivated to combine the references in this manner to avoid ligation of the first and second primers and to avoid self ligation of the primers. Applicants respectfully disagree with both assertions. First, Pederson does not teach the combination of one adaptor blocked at the 5' end and one adaptor blocked at the 3' end. There is nothing in Pederson to suggest a pair of adaptors with this combination or that this combination would be preferable to any other possible combination. The combination of one adaptor that is blocked at the 3' end and capable of ligation at the 5' end and a second adaptor that is blocked at the 5' end and capable of hybridization at the 3' end, as required by the claims, allows formation of a 5'-adaptor-fragment-adaptor-3' strand that can serve as a template for amplification (see Figure 1 of the application) and prevents amplification of the fragments that have the same adaptor on both ends. Other combinations would have different results. For example, if both adaptors were blocked at the 5' end, then only 5'-adaptor-fragment-3' templates would be formed. If both adaptors were blocked at the 3' end, then only 5'-fragment-adaptor-3' templates would be formed. Neither would serve as template for exponential amplification.

In addition, Applicants respectfully disagree with the Examiner's assertion that one of skill in the art would have been motivated to modify the method of McCasky Feazel *et al.* to include a first adaptor blocked from ligation at a 5' end and a second adaptor blocked from ligation at a 3' end. The Examiner indicates that the motivation to do so would be to prevent the first adaptor from ligating to the second adaptor and to block self-ligation. Applicants disagree with the assertion that the combination would result in blocking the first adaptor from ligating to the second adaptor. Because the 5' end of one adaptor and the 3' end of the other adaptor are not blocked those ends can ligate, resulting in ligation of the first adaptor to the second adaptor to generate a template for exponential amplification. Applicants also dispute the assertion that one of skill in the art would be motivated to block self-ligation in order to improve the efficiency of ligation. Unlike the situation where a fragment is being ligated into a vector and the ends of the vector may be treated to block self ligation because there is a preference for the ends of the vector to ligate together in a unimolecular reaction, the ends of the fragments and the adaptors are equivalent substrates for ligation so self-ligation of the adaptors does not significantly impact

the efficiency of ligation of the adaptor to the fragments. The cost of blocking would far outweigh any efficiency that might be gained by reducing adaptor self-ligation.

Also, the method of McCasky Feazel relies on the identification of differentially amplified nucleic acids. One of the most significant forms of variation that result in differentially amplified nucleic acids according to the methods of McCasky Feazel *et al.* is variation that changes bases in or immediately next to a restriction site. Eliminating from the analysis all fragments cut on both ends by the same enzyme would needlessly reduce the possible number of variants that could be identified. For example, when the enzymes used are *MseI* and *EcoRI*, most of the fragments will be cut on both ends by *MseI*. A deletion or an insertion of an *MseI* site from a fragment cut on both ends by *MseI* would not be detected as a differentially amplified fragment if the *MseI* adaptor was blocked from ligation at either the 5' or 3' end. Combining adaptors that block amplification of the fragments cut on both ends with the same restriction enzyme would eliminate the detection of a large number of polymorphisms and render the method taught by McCasky Feazel *et al.* unsatisfactory for its intended purpose.

In paragraph 7 claims 29-31 are rejected under 35 USC § 103(a) over McCasky Feazel *et al.* in view of Pederson and in view of Guire *et al.* (U.S. Patent No. 6,514,768). As discussed above McCasky Feazel *et al.* in view of Pederson fails to teach digestion with a first and second enzyme and ligation of a first adaptor blocked at the 5' end and a second adaptor blocked at the 3' end as required by the claims. Guire *et al.* fails to remedy the deficiencies of McCasky Feazel *et al.* and Pederson.

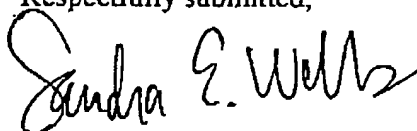
In paragraph 8 claims 35-39 are rejected under 35 USC § 103(a) over McCasky Feazel *et al.* in view of Pederson and further in view of Short *et al.* (U.S. Patent No. 6,238,884). As discussed above McCasky Feazel *et al.* in view of Pederson fails to teach digestion with a first and second enzyme and ligation of a first adaptor blocked at the 5' end and a second adaptor blocked at the 3' end as required by the claims. Short *et al.* also fail to teach a mechanism to preferentially amplify those fragments that are ligated to both a first adaptor and a second adaptor. Therefore, Short *et al.* fails to remedy the deficiencies of McCasky Feazel *et al.* and Pederson. Applicants respectfully request withdrawal of the rejections.

## CONCLUSION

In view of the foregoing amendments and remarks, Applicants believe all pending claims are now in condition for allowance and should be passed to issue. If the Examiner feels that a telephonic interview would in any way expedite the prosecution and allowance of this application, please do not hesitate to call the undersigned at (408) 731-5768. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account 01-0431.

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Respectfully submitted,



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